Chronic natural killer lymphoproliferative disorders: characteristics of an international cohort of 70 patients

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Background: The 2008 World Health Organization (WHO) classification distinguishes three entities among the large granular lymphocytic leukemia (LGL leukemia): T-cell LGL leukemia (T-LGL leukemia), aggressive natural killer (NK) cell leukemia, and chronic NK lymphoproliferative disorders (LPD), the later considered as a provisional entity. Only a few and small cohorts of chronic NK LPD have been published.

Patients and methods: We report here clinicobiological features collected retrospectively from 70 cases of chronic NK LPD, and compared with those of T-LGL leukemia.

Results: There were no statistical differences between chronic NK LPD and T-LGL leukemia concerning median age (61 years [range 23–82 years]), organomegaly (26%), associated autoimmune diseases (24%), and associated hematological malignancies (11%). Patients with chronic NK LPD were significantly less symptomatic (49% versus 18%, P < 0.001) and the association with rheumatoid arthritis was more rarely observed (7% versus 17%, P = 0.03). The neutropenia (<0.5 × 10⁹/l) was less severe in chronic NK LPD (33% versus 61%, P < 0.001) without difference in the rate of recurrent infections. STAT3 mutation was detected in 12% of the cohort, which is lower than the frequency observed in T-LGL leukemia. Thirty-seven percent of the patients required specific therapy. Good results were obtained with cyclophosphamide. Overall and complete response rates were, respectively, 69% and 56%. Overall survival was 94% at 5 years.

Conclusion: This study suggests very high similarities between chronic NK LPD and T-LGL leukemias. Since chronic NK LPD is still a provisional entity, our findings should be helpful when considering further revisions of the WHO classification.

Key words: leukemia, large granular lymphocytic/pathology, natural killer cells

introduction

Large granular lymphocytes (LGL) are cytotoxic cells that play an integral role in innate immune response. They derive from T (CD3+) or natural killer (NK, CD3− membrane) lineages. There are normally 0.25 × 10⁹ LGL/l in peripheral blood [1]. LGL leukemia is characterized by a clonal proliferation of cytotoxic lymphocytes. In 1985, LGL leukemia was identified as a clonal proliferation characterized by tissue invasion of bone marrow, liver, and spleen [2]. It was then recognized as a distinct entity in the French–American–British classification. The spectrum of LGL...
leukemias has changed over the last years. The World Health Organization (WHO) classification published in 2008 [3] distinguishes three entities: T-cell granular lymphocytic leukemia (T-LGL leukemia), chronic lymphoproliferative disorder (LPD) of NK cells (chronic NK LPD, defined as a provisional entity), and aggressive NK cell leukemia. T-LGL leukemia and chronic NK LPD are defined by a sustained (>6 months) LGL proliferation with a LGL count >0.5 × 10⁹/l [4]. T-LGL leukemia typically displays a terminal effector activated T-cell phenotype CD3+CD8+/CD45RA+/CD57+/CD62L− [4]. Monoclonality is demonstrated by a clonal T-cell receptor TCRγ gene rearrangement. In case of chronic NK LPD, LGL shows a CD3−/CD8+/CD16+/CD56+ phenotype. Monoclonality cannot be proved, but killer cell immunoglobulin-like receptor (KIR) repertory analysis may show a dysregulation in KIRs expression on LGL: this skewed KIR repertory expression may in a certain way contribute to assess clonality [5, 6]. T-LGL leukemia and chronic NK LPD are both chronic and indolent diseases characterized by neutrophilia associated with autoimmune conditions such as rheumatoid arthritis. T-LGL leukemia may occur even more rarely in a more aggressive form: patients present with massive hepatosplenomegaly, gastrointestinal and central nervous system involvement, B symptoms, and cytopenias [7]. Aggressive NK-LGL leukemia is mostly reported in Asia or Central America, and very rarely in Western countries. Prognosis is very poor despite treatment with polychemotherapy, and bone marrow transplantation. EBV infection plays an important role in physiopathogenesis, which probably explains its geographical distribution [8, 9].

Only a few series of chronic NK LPD have been published [10–16]. They suggest clinical and hematological similarities with T-LGL leukemia. Furthermore, these two entities share common physiopathological features. In 2012, STAT3 mutation was found in about 40% of T-LGL leukemia and 30% of chronic NK LPD [15, 17], unifying pathogenesis between both entities. We report here clinicobiological data from the largest cohort of chronic NK LPD.

patients and methods

patients

Patient data were collected from the French national LGL proliferation registry setup at Rennes University Hospital and from Italian and US LGL leukemia registry held in Padova Hospital, and University of Virginia Cancer Centre, respectively. Thirty-three patients had previously been reported from US (N = 13) and French (N = 22) registries [14, 15, 18]. All patients gave their informed consent to the use of their medical records for the benefit of research.

diagnosis criteria

The diagnosis was based on a peripheral long-lasting (>0.5 × 10⁹/l and >6 months) LGL proliferation with NK immunophenotyping, following recommended diagnostic criteria [4]. In the case of a circulating NK LGL count <0.5 × 10⁹/l, the diagnosis was established based on clinical or hematological features, and LGL bone marrow infiltration.

exclusion criteria. Patients with a short follow-up (<6 months) were excluded, according to the definition [4]. Aggressive LGL NK leukemias characterized by aggressive clinical course, pancytopenia, and massive bone marrow infiltration were excluded from this study [8, 9].

flow cytometry analysis. NK subtype was defined by flow cytometry analysis using common surface markers showing CD3−/CD16+, or CD56+ pattern. Samples analysis studied at least the following markers: CD3/CD8/CD16/CD56/CD57. Analysis of NK receptors expression was carried out on 38 patients, using the following markers: CD94, NKGD2, NKGD2A, NKBP1, CD158a, CD158b, and CD161. Natural cytotoxicity receptors (NCRs, Nkp46, Nkp44, and Nkp30) were studied in 9 patients. In 24 cases, Vβ repertory was studied by flow cytometry analysis (IOTest Beta Mark TCR Vβ repertory kit, Beckman Coulter, Miami, FL), demonstrating no evidence for clonality.

molecular analysis

TCRγ gene rearrangement was studied by polymerase chain reaction in 25 cases and demonstrated no evidence for clonality [19]. STAT3 SH2 domain sequencing was carried out in 40 of the 70 patients. French laboratory used Sanger sequencing technique, whereas a DNA tetra-primer amplification–rearrangement mutation system (ARMS) assay was carried out in the Italian and American laboratories following the instructions previously published [15].

response criteria

Response to treatment evaluation was based on periodical clinical and biological hematological examinations. Complete response (CR) was defined as a complete resolution of clinical symptoms and complete normalization of blood count [i.e. hemoglobin (Hb) >11 g/dl, platelets (Pl) >150 × 10⁹/l, absolute neutrophils count (ANC) >1.5 × 10⁹/l, and lymphocytosis (Ly) <4 × 10⁹/l], Partial response (PR) was considered as an improvement in blood counts (i.e. Hb >80 g/l, Pl >50 × 10⁹/l, ANC >0.5 × 10⁹/l) and in transfusion requirements [14]. Phenotypic response was not assessed in this retrospective multicentre study. Treatment failure was defined as any response not meeting the above criteria 2 months after treatment introduction.

statistical analysis

All the statistical analyses were carried out on Statistical Analysis System software version. The Kaplan–Meier method was used to estimate survival rates. Initial characteristics were investigated for their correlation to the overall survival (OS) using the log-rank test and a Cox multivariate model (P < 0.05).

The characteristics of this cohort was compared with the large French series of 201 T-LGL leukemia previously published [14]. The significance of the differences observed was tested using the χ² test and Fisher’s exact test, when appropriate.

results

Seventy patients diagnosed with a chronic NK LPD between January 1984 and March 2012 were included: 37 from France, 20 from Italy, and 13 from the United States. Median age at diagnosis was 61 (range 23–82) years. Sex ratio M/F was 1.4 (41/29). Symptoms at diagnosis were as follows: fatigue and/or B symptoms (30%), autoimmune–associated disease (24%), splenomegaly (14%), recurrent infections related to mild or severe neutropenia (14%), and peripheral neuropathy (3%). Most of the recurrent infections involved oral cavity, and more rarely skin, GI, or respiratory tract.

Nine patients (13%) had a previous history of solid neoplasm, diagnosed several years before chronic NK LPD. There was an associated hematological malignancy in 8 patients (11%). Four of them were diagnosed concomitantly to chronic NK LPD, four during the follow-up, after a median time of 3 years (2–12 years), whereas two patients had been diagnosed as multiple myeloma.
and polycythemia, 10 and 5 years before chronic NK LPD, respectively. In the first patient, multiple myeloma had previously been treated by oral melphalan and corticosteroids stopped 3 years before the diagnosis of chronic NK LPD. Eleven patients (15%) presented autoimmune cytopenia: immune thrombocytopenic purpura (n = 5), hemolytic autoimmune anemia (n = 5), and autoimmune neutropenia (n = 1). Clinical characteristics are depicted in Table 1.

Ly (>4 × 10⁹/l) was present in 55% of cases. Median Ly and LGL count were, respectively, 4.5 × 10⁹/l (2.1–6.5 × 10⁹/l) and 2.1 × 10⁹/l (0.8–4.1 × 10⁹/l). Nineteen patients (29%) and 4 patients (6%) had <1 × 10⁹/l and 0.5 × 10⁹/l circulating LGL, respectively. Half of the patients (51%) did not show any cytopenia at diagnosis. Neutropenia (ANC <1.5 × 10⁹/l) was observed in 23 of them (29%); it was severe (<0.5 × 10⁹/l) in only 9% of the patients (6/70). Only 10 neutropenic patients presented recurrent infections, related to mild or severe neutropenia. Anemia (<110 g/l) was present in 18% of the cases, and eight patients (11%) required red blood cell transfusion. All hematological data are reported in Table 2.

Marrow LGL infiltration was studied in 70% of the cohort. Median rate of infiltration was 25% (from 15% to 70%). It was diagnosed either on bone marrow smear examination associated to flow cytometry analysis, or on bone marrow biopsy specimens associated to immunostaining (clusters of more than 6 CD3ε+, CD56+, Granzyme B+, perforin + cells [20]).

In all cases, LGL immunophenotype was CD3−/CD16+ or CD56+. CD57 was expressed in 46/64 cases (72%), and CD7 in 28/33 cases (85%). CD8 was detected in 57% of the cases. High expression of the lectin receptor CD94 was observed in 40/44 patients (91%). KIR repertory analysis was carried out in 38 patients (54%); 55% of them expressed less than two inhibitory receptors. KIR3DL1 (NKB1) was expressed in only 26% of the cases, and KIR2DL1 in 31% of the cases. Lectin subtype inhibitory receptor NKG2A (CD159a) was present in 73% of the cases. NCRs were analyzed in only nine patients. Nkp44 was never expressed and Nkp30 only in 1 of 9 patients (11%), whereas Nkp46 was detected in 66% of those patients (supplementary Data S1, available at Annals of Oncology online).

STAT3 mutation status was studied in 40 patients (57%). Only five patients presented a SH2 domain activating mutation (12.5%): four D661Y mutations, and one Y640F mutation.

Twenty-six patients (37%) received specific therapy related to chronic NK LPD. They received a median of 2 lines of treatment.

Table 1. Clinical manifestations and associated diseases of the 70 NK chronic lymphocytosis, compared with T-LGL characteristics [14]

<table>
<thead>
<tr>
<th>Chronic NK-LGL lymphocytosis (N = 70)</th>
<th>T-LGL Leukemia, Bareau et al. (N = 201)</th>
<th>P &lt; 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median follow-up (months, min–max)</td>
<td>57.5 (2–324)</td>
<td>58</td>
</tr>
<tr>
<td>Age (years, min–max)</td>
<td>61 (23–82)</td>
<td>59</td>
</tr>
<tr>
<td>Sex ratio M/F (% men)</td>
<td>41 (29–59%)</td>
<td>45%</td>
</tr>
<tr>
<td>Symptoms at diagnosis (%)</td>
<td>51% (36–70)</td>
<td>82%</td>
</tr>
<tr>
<td>Tumoral syndrome</td>
<td>26% (18–70)</td>
<td>29%</td>
</tr>
<tr>
<td>Splenomegalya</td>
<td>14% (10–70)</td>
<td>24%</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>13% (9–70)</td>
<td>10%</td>
</tr>
<tr>
<td>Polyadenopathy</td>
<td>6% (4–70)</td>
<td>6%</td>
</tr>
<tr>
<td>Fatigue</td>
<td>26% (18–70)</td>
<td>7%</td>
</tr>
<tr>
<td>Recurrent infections</td>
<td>14% (10–69)</td>
<td>23%</td>
</tr>
<tr>
<td>Associated autoimmune diseasesb</td>
<td>24% (17–70)</td>
<td>33%</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>7% (5–70)</td>
<td>17%</td>
</tr>
<tr>
<td>Unclassified arthritis</td>
<td>14% (6–70)</td>
<td>8%</td>
</tr>
<tr>
<td>Vascularitis</td>
<td>4% (3–70)</td>
<td>3%</td>
</tr>
<tr>
<td>Polymyositis</td>
<td>3% (2–70)</td>
<td>0%</td>
</tr>
<tr>
<td>Hashimoto thyroiditis</td>
<td>1% (1–70)</td>
<td>1%</td>
</tr>
<tr>
<td>Peripheral neuropathy</td>
<td>3% (2–70)</td>
<td>1.5%</td>
</tr>
<tr>
<td>Autoimmune cytopenias</td>
<td>14% (10–69)</td>
<td>7%</td>
</tr>
<tr>
<td>Idiopathic thrombocytopenic purpura</td>
<td>7% (5–69)</td>
<td></td>
</tr>
<tr>
<td>Autoimmune hemolytic anemia</td>
<td>6% (4–69)</td>
<td></td>
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<tr>
<td>Autoimmune neutropenia</td>
<td>1% (1–69)</td>
<td></td>
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<tr>
<td>Pure red cell aplasia</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Associated solid neoplasms</td>
<td>13% (9–70)</td>
<td>5%</td>
</tr>
<tr>
<td>Associated hemopathiesb</td>
<td>11% (8–70)</td>
<td>8%</td>
</tr>
<tr>
<td>B-cell lymphoma</td>
<td>3% (3–70)</td>
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<tr>
<td>Myeloma</td>
<td>1% (1–70)</td>
<td></td>
</tr>
<tr>
<td>MDS</td>
<td>3% (2–70)</td>
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<tr>
<td>AML</td>
<td>3% (2–70)</td>
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<tr>
<td>CML</td>
<td>1% (1–70)</td>
<td></td>
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<tr>
<td>Polycythemia</td>
<td>3% (2–70)</td>
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</tbody>
</table>

Statistically significant differences (P < 0.05) are reported in the right column, with the corresponding P value.

*aSplenomegaly was detected on clinical examination.

*bSome patients had several associated hemopathies.

MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; CML, myeloid chronic leukemia.
Indications for treatment were as follows: one or more cytopenias (56%), autoimmune-associated disease (39%), and fatigue (5%).

Oral cyclophosphamide (100 mg/day) was administered in 17 patients (53%), mainly as a first-line therapy (74% of the cases). Indications were cytopenias (11/17; 65%), associated autoimmune disease (5/17; 29%), and fatigue (1/17; 6%). Mean treatment duration was 7.5 months (5–10 months). It was associated with oral corticosteroids in half of the cases (median dose of 1 mg/kg/day). Overall response rate (ORR) was 69% (11/16), including 56% of CR and 13% of PR, with a median duration of 29 months (16–38 months). Median time to response was 4 months (3–8 months). There was only one relapse observed 18 months after cessation of treatment. One patient with pancytopenia responded durably to cyclophosphamide after failing three previous lines of treatment including methotrexate (MTX).

MTX was delivered in 11 patients (49%) as a first (n = 6) or as a second-line therapy (n = 5). Indication of treatment was: associated autoimmune disease (n = 6), cytopenia (n = 4), and a specific pleurisy (n = 1). Median dose was 12.5 mg/week. It was always associated to corticosteroids (5–20 mg/day). Median duration of treatment was 12 months (10–13 months). ORR was 54% (6/11), mainly PR (36%), with a median duration of 9.5 months (7–21 months). Time to response was 5.5 months (3–8 months). Only one relapse occurred 5 months after cessation of treatment.

The other treatments were very heterogeneous and administered in only a few patients: polychemotherapy (n = 3), ciclosporin (n = 2), and prednisone (n = 2). Splenectomy was carried out in three patients. They mainly didn’t achieve any response, except one case of chronic NK LPD associated to a B lymphoma presenting with pancytopenia, which was successfully treated by polychemotherapy.

In univariate and multivariate analyses, none of the patient clinical or biological characteristics reported in Tables 1 and 2 was correlated to overall response rate.

With a median follow-up of 58 months, the 5-year and 10-year OS was, respectively, 94% and 84% (supplementary Data S2, available at Annals of Oncology online). Eight patients died during the follow-up (11%): only one patient died because of the chronic NK LPD (infectious complication on the onset of severe neutropenia). The other patients died because of an associated hematological malignancy (n = 5), or cardiac failure (n = 2).

In univariate analysis, only age at diagnosis (P = 0.006), tumoral syndrome (P = 0.002), splenomegaly (P = 0.0001), B symptoms at (ranged from 1 to 5). Indications for treatment were as follows: one or more cytopenias (56%), autoimmune-associated disease (39%), and fatigue (5%).

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KIR expression on NK LGL has been demonstrated [5, 6, 24] not express TCR, clonality cannot be assessed, except by human detection of a dominant Vβ TCR repertoire. As NK cells do reaction (PCR) analysis and highly suggested by Flow Cytometry smaller cohorts previously published [10]. We finally compared this chronic NK LPD cohort to the T-LGL leukemia cohort we previously published [14] (Tables 1 and 2). There were significantly more asymptomatic patients (49% versus 18%, P < 0.05) in this cohort compared with T-LGL leukemia. The incidence of RA was lower (7% versus 17%, P < 0.05). The degree of neutropenia was less severe in NK subtype. It probably explains why infections rate was higher in the T-LGL leukemia subtype, although nonsignificant (14% versus 23%, P > 0.1). Chronic NK LPD were more frequent associated with autoimmune cytopenias (15% versus 7%, P < 0.05), and solid neoplasms (13% versus 5%, P < 0.05). There was no significant difference in 5-year OS (89% versus 94%, P = 0.4).

**discussion**

We present here the largest cohort of chronic NK LPD published so far. Even if data were retrospectively collected, this study provides valuable information about this provisional entity defined in the 2008 WHO classification [3].

Our results extend and confirm those of the chronic NK LPD smaller cohorts previously published [10–16]. Chronic NK LPD is an indolent pathology and the main patient characteristics are splenomegaly (30%), autoimmune diseases (24%) and autoimmune cytopenias (15%). About half of them are asymptomatic.

Comparing our data with the cohort of T-LGL leukemias, we previously published [14], we show that chronic NK LPD share many common features with T-LGL leukemia. We also observe some differences. Asymptomatic patients are more frequent in NK subtype (49% versus 18%, P < 0.05). Association with RA is less frequent in chronic NK LPD (7% versus 17%, P < 0.05). The incidence of solid tumors is higher in NK subtype. The degree of neutropenia is also significantly lower but the incidence of septic complications is not statistically different: we underline that the rate of recurrent infections (23%) was lower in the French series when compared with that observed in the previous cohorts of T-LGL leukemia: 39% in Loughran’s series [21] and 56% in Semenzato’s study [22]. These two entities present with a chronic evolution and disease related death is a rare event. In this cohort, only one patient died from chronic NK LPD (8%), due to septic shock. OS is not statistically different (5-year OS: 94% versus 89% for T-LGL leukemia, P = 0.4).

Monoclonality is an important part of the definition of T-LGL leukemia. It is determined by TCRγ polymerase chain reaction (PCR) analysis and highly suggested by Flow Cytometry detection of a dominant Vβ TCR repertoire. As NK cells do not express TCR, clonality cannot be assessed, except by human androgen receptor-X chromosome assay (HUMARA) that is limited to female patients [23]. In the last 15 years, dysregulated KIR expression on NK LGL has been demonstrated [5, 6, 24–26], related to an abnormal methylation pattern of the KIR genes promoters [5]. We confirm in this cohort that LGL NK cells express a skewed KIR repertory as they all lack at least one inhibitory receptor, some of them even lacking all the tested KIR as previously described [25]. High frequency of KIR-activating receptors expression in LGL NK cells compared with healthy donors has already been suggested by genotypic analysis [6, 27, 28]. In those studies, flow cytometry analysis was less extended or concerned smaller number of cases.

There is no formal consensus concerning therapy in LGL leukemias, because of the lack of prospective trials. We published treatment recommendations based on retrospective studies treating almost exclusively cases of T-LGL leukemia [4, 18]. MTX and cyclophosphamide are considered as the 2 main first-line options. We confirm this statement as both drugs induce good response rate (54% and 69% ORR, respectively) in this NK LPD cohort, either in neutropenic or anemic patients. Physiopathogenesis of LGL leukemia is very complex. Role of exogenous agents, in particular viruses, is suggested [4, 21]. Skewed KIR repertory characterized by the increase expression of activating KIRs suggests that these receptors may be involved in the priming of the NK proliferation. Recognition of virally infected cells through activating KIRs may participate to the clonal expansion of the LGL NK cells. Recently, STAT3 and STAT5 somatic mutations have been identified in T-LGL leukemia and chronic NK LPD. These mutations contribute to induce constitutive activation of JAK/STAT3 pathway [12, 15, 29] and maintain leukemic LGL expansion and survival. These findings strongly suggest common specific pathway in T-LGL leukemia and chronic NK LPD. We found a lower rate of STAT3 mutation in our chronic NK LPD cohort than previously published (12% compared with 30% in Jerez study) [15]. In that study, STAT3 mutation status was associated with a higher frequency of B symptoms and RA at diagnosis, which could not be evaluated in our cohort considering the poor number of STAT3 mutated patients. Whether or not STAT3 mutation is less frequently associated to chronic NK LPD remains to be evaluated on prospective study. Teramo et al. proved that even if mutation was absent, JAK/STAT3 pathway was alternatively activated in T-LGL, especially by the underexpression of SOCS3 a negative feedback inhibitor. Thus, in nonmutated patients, high levels of activated phosphorylated form pSTAT3 were detected, and inhibition of STAT3 resulted in restoration of tumor cells apoptosis [29]. So, despite the lower rate of STAT3 activating mutation in our cohort, the role of JAK/STAT3 pathway in chronic NK LPD pathogenesis cannot be excluded and should be evaluated.

Our study helps clinicians and biologists to better understand this provisional entity declined in the 2008 WHO classification. It confirms some differences in terms of clinicobiological presentation and prognosis compared with aggressive NK cell leukemia. NK chronic LPD shares in contrast many common features with T-LGL leukemia, especially concerning clinical features, pathogenesis, prognosis, and response to immunosuppressive therapy. We propose to reconsider chronic LGL proliferations as a unique entity regardless the LGL lineage.

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**references**